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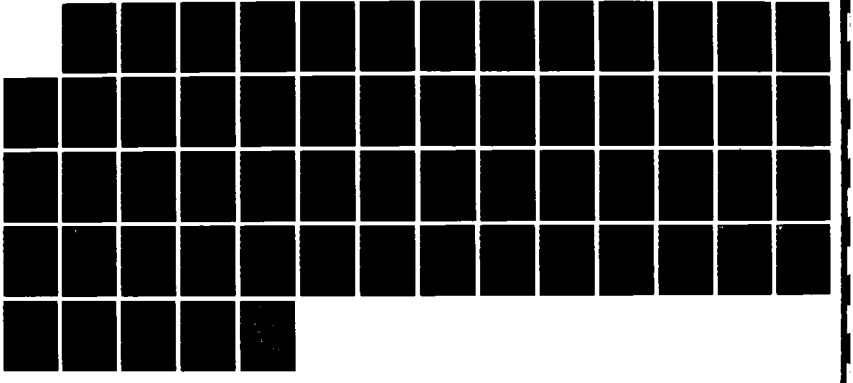

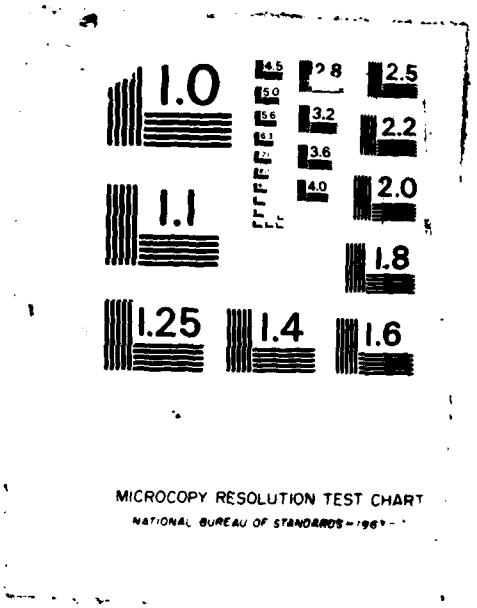

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Visual System Neural Responses to Laser Exposure from Local Q-Switched Pulses and Extended Source CW Speckle Patterns

Final Report

Myron L. Wolbarsht, Ph.D.

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The reports of retinal damage from exposure to short pulse laser energy without any visual perception have been reviewed. A series of experiments has been conducted in cats to test retinal function after such laser exposures using neurophysiological recordings from retinal ganglion cells. The receptive fields of the selected ganglion cells were in the area of laser exposure or immediately adjacent to it. Microsensor monitoring of retinal metabolic stability including pH and O_2 levels following laser exposure was also attempted. Vigorous neural responses have been recorded from retinal ganglion cells following suprathreshold lesion producing laser exposures within the ganglion cell receptive fields. These recordings were made from electrodes within the receptive fields or from nerve fibers at the optic disc. The disc recordings always showed responses from ganglion cells following supra-threshold stimulation levels sufficient to cause retinal lesions within the recep-tive field or in closely adjacent areas of the retina. These data suggest that

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18. retinal ganglion cell response laser induced metabolic changes

19. there should be perception of all retinal injuries other than those sufficient to ablate the optic nerve head and possibly even then. Suprathreshold laser exposures within the receptive field of the ganglion cell, in general, abolished the central responses of the ganglion cell leaving only the large peripheral response indicating that bipolar and other intermediate retinal cells were affected. Pilot studies showed that exposure to low power levels of large field CW laser speckle patterns did not appear to change the make-up of the receptive field. There were no definite conclusions about the effect of any types of laser lesions on metabolic levels within the retina as the nitrous oxide anesthetic inter- fered with the recordings of O_2 levels. The recordings of pH levels within the retina were unsuccessful possibly due to electrode shape. The many advantages that were found for recordings from neural fibers at the optic disc suggest that future experimental protocols should specify this approach, along with intensive investigation into the conditions necessary to make this approach successful more often.

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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A. BACKGROUND

Several laser workers have complained that they have received laser exposures resulting in injury, although they received little or no visual sensation at the time of the exposure. They explained that they did not realize that they had been exposed to a high and damaging abnormal level of laser energy, and furthermore, that there was no uncomfortably bright light which made them realize that they had been potentially injured. Documentation of this information by published reports is difficult, as most such injuries are not put in a public written form due to legal considerations. However, such anecdotal reports as do exist are sufficient to define the problem clearly. Many of the laser injuries in question occur from exposure to energy in the near infrared region. Nevertheless, even in this region, the threshold power or energy level for visual stimulation by laser exposures is still below that for injury. For example, Nd:YAG laser exposures at 1,064 nm give clearly discernible chromatic visual responses at power levels far below those necessary for retinal damage (Sliney et al, 1976). However, some injuries have followed multiple pulse exposures in the IR, in which no single pulse was above the threshold, but as the deleterious effects of multiple pulses in a train are seen to be somewhat additive, exposure to the entire train results in a suprathreshold level followed by retinal damage. In this case, the last pulses in the train may not have been visible as the injury threshold was reached. This loss of retinal response may have occurred in the damaged area, as well as in the surrounding non-damaged area

where the receptors are certainly stimulated by subthreshold direct exposure or scattered radiation, if not directly within an undamaged area.

It is also possible that the peculiar features of the speckle in a sub-injury threshold laser image may produce a chronic inactivation of the retinal response to prevent subsequent appreciation of a super-threshold exposure. Zwick and Beatrice (1978) and Zwick et al (1980) have indicated that exposure to speckle patterns at levels below those causing thermal damage results in a more or less permanent loss of chromatic sensitivity. This damage seems to be localized in the inner layers of the retina, as the ERG changes which accompany this loss (Zwick et al 1980, Zwick and Jenkins, 1981) are characteristic of changes in Mueller cell behavior (Karwoski and Proenza, 1977). Zwick and co-workers (1978, 1980) used laser exposures that included only extremely large retinal areas. Nevertheless, the same type of retinal inactivation may occur on a short-term basis in restricted retinal areas. This effect could lower the local sensitivity sufficiently to prevent appreciation (perception) of the full duration of a laser stimulus train.

Those documented case reports in which the retinal injury was not associated with light perception are quite variable.

Boldrey et al (1981) reported that an exposure from a Q-switched neodymium YAG at 1.06 μm with a 6 ns pulse and 10 pulses per second

produced a retinal injury. The patient felt a pop and pain, but did not have any sensation of light. It should be noted that sub-threshold neodymium laser pulses at 1,064 nm are reliably seen by almost all observers. For example, Sliney et al (1976) conducted a series of experiments on the perceived color of this type of laser exposure. Although most people saw it as colorless, some reported it as green. However, all saw it at approximately the same exposure level.

A retinal injury was caused by exposure to a collimated 858.5 nm beam originating from a dye cell shifted, frequency doubled Nd-YAG laser with 10 ns pulses repeated at 10 Hz. The patient reported that no light flash was seen, but suddenly loss of vision in one eye was noticed while the laser was operating (Sliney/NRL-1981).

As mentioned above, very few laser accidents are reported in the open literature in detail. Although many semi-public inquiries are made in the course of investigations concerning laser accidents, the possible litigation associated with almost all accidents prevents most cases of laser exposure involving functional loss (i.e., suprathreshold burns) from appearing in the open literature. Most of the reports on exposures from a red or infrared laser mention pain following the injury, and from the description of floaters, etc. show evidence of a retinal hemorrhage (see Boldrey et al, 1981 for review). Although in all cases there is some form of scotoma, this may or may not be associated with a persistent after-image.

The ever increasing number of laser exposures for clinical use in retinal therapy, mostly for diabetic retinopathy, furnishes some information. The reports from those patients who undergo panretinal photocoagulation are varied. The unsolicited description by the patient is either of pain or no sensation at all other than a flash of light. Pain is felt by relatively few from an argon laser, but much more often from the 670 nm krypton laser treatment. Also, the xenon arc photocoagulator, which has a large retinal image with a large total amount of heat, often has pain reported. However, in the clinical situation, the blink reflex is inhibited and the iris is immobile eliminating iris spasm, often the cause of the pain following bright light exposure. When the pain from this cause is eliminated, the light may not seem as bright or even may not be objectionable.

The halo surrounding the central portion of a diffraction limited image of a laser contains a considerable portion of the energy even in the case of a threshold exposure. If the energy in the first diffraction ring is only 5% of that in the center, this should be sufficient to elicit a visual response from those receptors which surround the laser damaged group in the center. Since the outer segments of the receptors are not exactly in the focal plane of the pigment epithelium, which is the thermal heat source, some out-of-focus broadening of the damage zone may be expected. Nevertheless, even outside of the approximately 0.02 mm retinal lesion formed by a diffraction limited image laser exposure, the ideal point spread function indicates that there is a large retinal area illuminated by at

least 1,000th of the peak intensity in the center. This level is certainly high enough to produce visual perception. As this area would be increased by any scattered light, it seems clear that a sizeable, non-injured group of photoreceptors are stimulated sufficiently to give a visual response during any damage level exposure from any visible laser. However, as the laser wavelength moves into the infrared, the normal response to be expected is not as obvious.

Several factors could prevent the information from leaving the retina along the optic nerve fibers after stimulation of the receptors. For example, in the macular region, if the laser is the proper wavelength to be absorbed by the macular pigment, xanthophll, a lesion can be formed in the outer plexiform layer at the same time and at the same power level as a lesion is formed in the photoreceptor outer segments and pigment epithelium. The blue line (488 nm) from an argon laser will cause this kind of double lesion (Wolbarsht and Landers, 1972; Landers et al, 1976; and Boybyes et al, 1973). If such a lesion in the inner retina was to form quickly enough, it would block the flow of information from receptors immediately underneath. However, each ganglion cell is connected to a large number of receptors through a large and complex receptive field. Thus, both the size and complexity of the receptive fields of most ganglion cells will still allow stimulation of ganglion cells in the damaged areas by peripheral receptors. The diffuse organization of the retina will allow information flow to proceed, so that the message from the receptors can reach the optic nerve and be transmitted back to the brain, initiating

perception. Nevertheless, it seems that a short laser pulse would act as a stimulus source for all receptors receiving sufficient photons. This kind of a stimulus will activate later neurons because of the large change in stimulus strength, although the stimulus parameters might not be accurately described, especially with respect to color. It seems reasonable, on the basis of our present knowledge, that the initial stimulus (in addition to some kind of after image) would certainly be perceived from a short laser pulse. Possibly, a long duration laser pulse could be delivered with a proper time temperature histogram to cause the receptors to die at the same time they were stimulated without, however, exceeding the visual threshold in some (but probably not all) surrounding receptors. This would be a minimal visual stimulus case. Numerous studies have indicated that there are detectable changes in the retinal anatomy from pulsed laser exposures in the infrared below those giving ophthalmoscopically visible loss of function. The widespread use of many kinds of laser equipment operating in the near infrared in multipulse modes could result in large amounts of subthreshold laser exposures to Army personnel. The work of Adams et al (1972) has shown that minimal pathological changes in the retina can result from single pulsed laser exposures. Although this type of minimal damage is far below that demonstrated to result in a functional loss, it is possible that the cumulative results of such exposures might result in physiological changes in the eye, ultimately leading to functional loss.

The physiological changes resulting from suprathreshold laser exposures indicate alterations in distribution of oxygen tension within the retina following laser exposure due to changes in metabolism in the various layers of the retina. For example, therapeutic laser photocoagulation results in widespread destruction of the photoreceptor layer allowing choroidal oxygen to diffuse into the inner layers of the retina (Wolbarsht and Landers, 1980). Perhaps, even without widespread destruction of the photoreceptor layer, multiple exposures might lower the metabolic activity of the photoreceptor cells, the rods, and cones, and compromise their metabolism, resulting in a lower rate of oxygen consumption. This would allow additional oxygen to diffuse into the inner retinal layers. Autoregulation of tissue oxygen levels would then reduce the retinal circulation flow rate which could result in a buildup of metabolic waste products; CO_2 and lactic acid, thus, could lower the normal pH, possibly leading to the degeneration of the inner layers of the retina as seen in retinitis pigmentosa (Wolbarsht & Landers, 1980; Stefansson et al, 1981 a). Those are all long-term effects with a delay of weeks, months, or even longer. Thus, if they occurred at all, they would not be quick enough to change the visual perception of an isolated focal laser exposure. The changes in ERG responses mentioned by Zwick and Beatrice (1978) from a speckle pattern may have a similar chronic degenerative effect on the inner retina, although confined to the Mueller fibers as indicated by the ERG changes (Karwoski and Proenza, 1977). This whole effect may be due to increased synaptic activity in the inner plexiform layer due to the

speckle pattern. This activity may lead to metabolic changes and, if continued, pathological ones.

B. PRESENT WORK

The site of a laser burn in the pigment epithelium and adjacent photoreceptors and the relative circulatory and metabolic independence of this part of the retina from the inner neural layers both suggest that even the destruction of the photoreceptors will not disturb the function of the overlying neural retina. It has also been suggested that sufficient diffracted or scattered laser radiation in the portion of the retina surrounding the thermally damaged sufficient scattered light will generate a neural signal following the laser exposure. This information will be passed on through the inner retinal neural layers up into the optic nerve and on to the higher visual centers. Thus, it is almost certain that perception of a laser exposure will take place, and that the perception will be of a source larger than a diffraction limited source used to produce the lesion. Nevertheless, this signal may be too weak to be seen in the presence of any significant ambient light. In the present series of experiments, measurements of the neural function have been used to test whether, in fact, the retinal neural layers are stimulated, and if so whether a signal about the laser exposure reaches the retinal ganglion cells and is passed on to the higher visual centers. This should indicate that perception of a laser stimulation takes place.

Even though the visual senstivity is low to wavelengths of 1,060 or 1,064 nm from the neodymium glass or YAG lasers, nevertheless, the diffraction limited image of this source does produce sufficient energy away from the injury zone of the diffraction image to give significant visual information of the laser source. In the present series of experiments, the latency of the ganglion cell response has been measured following the almost certain death of the photoreceptor cells in the central of the lesion.

C. METHODS

External recordings from retinal ganglion cell bodies or their nerve fibers at the optic disc have been used to monitor the exposure sensitivity of the retina in the position of the laser image and to document the exposure condition which caused certain types of response. Intraocular metal electrodes have been used to monitor the nervous activity from the retinal ganglion cells. The structure of the receptive field of the ganglion cells have been mapped using the techniques of Wagner et al (1960) and Wolbarsht et al (1985). Responses to test sub-threshold laser exposures have been determined. Both sub-threshold and markedly super-threshold exposures have been used to determine whether markedly super-threshold exposures inhibit information flow, and thus, give a poor or absent perception of laser exposure when threshold level laser exposures give an unmistakable signal.

The present series of exposures have used single Q-switched laser pulses. The cumulative effects of multi-pulses have not been studied sufficiently to yield data for this report.

1. Laser Exposure and Optical Stimulation

Optical stimulation has been provided by a Maxwellian view system adapted from the two independent channel systems similar to that described by Wagner et al (1980) but modified with interference filters substituted for the monochrometer as described in Crocker et al (1980). The two channels have been used with either interference filters or broad band gelatin filters (Wratten Type Eastman Kodak Company, Rochester, New York) to furnish chromatic adaptation as used in identification of the type of the ganglion cells and characterization of the receptive field properties. The beams from the two channels can be combined so that they can pass as a single beam to a flat face corneal contact lens in the center of the fully dilated animal pupil. In this configuration, a focused, demagnified (10 to 1) image of the optical stimulator apperture is formed on the retina. Under these conditions, approximately 10 either side of center of the image of the beam can be stimulated on the retina directly. The selected retinal ganglion cells have been identified before and after subthreshold laser exposures to determine how the response has changed following a supra-threshold laser exposure. Maxwellian view optics have been used to allow sufficient intensitiy of chromatic narrow-band stimulating light to characterize easily and quickly the performance of

selected ganglion cells with a high degree of precision. The field aperture of the optical stimulator can form an image on the retina as small as 0.02 mm. Previous work has indicated that this is a very reliable figure, as it has been compared with known size of probes inserted into the eye and placed at the same place as the test pattern on the retina. Both laser exposure and stimulus beams have been approximately normal to the retina eliminating any change in stimulus response from the Stiles-Crawford effect. In order to achieve the Maxwellian view, a flat-face contact (or gonial) lens has been used on the cornea to eliminate that refracting surface and to assist in visualizing the posterior pole of the eye for long periods of time. The flat face contact lens was soft and highly oxygen permeable. Flowing well oxygenated solutions around the edge of this soft contact lens insured that the cornea remained clear during the course of the experiment.

The laser beams for lesion formation were introduced through a third channel and located and focused with a coaxial, low power (0.5 mW) helium-neon laser indicator beam attenuated in the minimum stimulus. The neodymium-glass laser system used for lesion formation has a 0.5 cm x 7.5 cm neodymium-glass rod and is Q-switched. It has a primary wavelength of 1,060 nm and can be frequency doubled. The beam is 5 mm in diameter at the cornea, and thus no artificial pupil is needed. In the present series of experiments, mode selection has not been used to give the minimum size image, but beam expansion and

constriction with mode selection by aperture position can be inserted if desired.

The calibration has been carried out with a calorimeter and a PIN photodiode to show the shape of the pulse. The absence of outstanding hot spots has been documented by photographing the raw beam.

Exposure to coherent light speckle patterns was made prior to the electrophysiological experiments in the animal quarantine room by illuminating one wall in a 2 meter circle by a 2 watt argon laser (488-514 nm). Changes in the animal care regulations necessitated changes in the exposure location causing delays in initiation and level of the coherent light exposure. The exposure level for 514 nm used by Zwick et al for rhesus monkeys was 12.5 uW/cm^2 over a 55° sector of the retina with an exposure duration of 20 hours (10 x 2 hr/day) for a dose of approximately 2 Joules. Our argon laser (488-514 nm) exposure dose was not sufficient to reach the threshold values to cause the changes in the retina observed by Zwick et al (1980) before the experimental electrophysiological recording program had been terminated. The lowered reflectivity of the new exposure room walls in the blue-green (488-514 nm), 0.41, as against 0.74 in the original, and the larger size of the room made it more difficult to get sufficient angular coverage and adequate exposure. Ganglion cell recordings were made on 2 animals before the calculated threshold change was reached. The total calculated exposure of each of the test animals was at 2.9 uW/cm^2

for 20 hours (0.14 J), less than 1/5th of that required by Zwick et al (1980) to achieve changes in neural function.

During three experiments, acute exposure of coherent light during electrophysiological recordings was made in order to determine if any neural effects could be found from a helium neon laser exposure at the 1.0 mW level which was spread over a retinal area approximately 2 mm in diameter for approximately 600 s. The retinal irradiance was 22 mW/cm^2, far above the brightness used by Zwick et al (1980), but the total dose (0.013 J) was much less.

2. Electrophysiological Techniques

Extracellular recordings have been made from retinal ganglion cells and optic nerve fibers from the intact eyes of adult cats. The electrophysiological recording equipment has been described previously in detail (Crocker et al, 1980).

Tungsten wire, electrolytically sharpened electrodes similar to those described by Levick (1972), were used in conjunction with a FET amplifier input stage. The amplified signal was put through a delay line filter to allow discrimination of two or more kinds of impulses, so that different ganglion cells could be used simultaneously for testing. However, in most cases, responses from single cells were isolated. The electrode and carrier were inserted into the eye through the region of the pars plana, as in conventional vitrectomy surgery.

The electrode was then placed against the retina under visual observation through the anterior part of the eye. When the electrode was positioned in the selected part of the retina, it was moved forward until it just penetrated the internal limiting lamina. Some recordings were made externally from ganglion cell bodies or axons near the optic disc. In usual types of recording, most of the electrode positions are at or near the center of the receptive field of the ganglion cell, as there are often mechanical motions of the electrode due to laser exposure; recordings were also made from the ganglion cell axons where they enter the optic disk. This was done in the same fashion by advancing the electrode to the rim of the disk. Previous experiments show the recording made at the disk are from the same kind of cells as are found by cell body or nearby axon recording. The recording at the optic disk are stable and seemed capable of continuing from the same neuron after laser exposure to a portion of the retina in the receptive field of that ganglion cell.

However, it was much more difficult to find cells at the disk, and recording times are much shorter, although some recordings were made with a total duration of several hours. The technique to achieve these long lasting recordings from the disk is most useful for markedly suprathreshold exposures, as data can be more reliably collected from them.

3. Data Acquisition and Analysis

The data points were determined for the light sensitivity of the ganglion cell, as well as its other parameters by a constant response technique. This is in order to ensure that changes in the baseline response from the ganglion cell could be detected during or following laser exposure. The ON/OFF response characteristics were documented, as well as the chromatic responses of the cell and its receptive field organization into center and surround. Both rod and cone contributions were identified in order to determine, if possible, which of the receptors gives rise to any response to the laser exposure.

4. Measurement of the O_2 and pH Level in the Eye

The technique for the measurement of the O_2 potentials has been described in detail previously (Stefansson et al, 1981(b); Landers, 1978). It was planned to measure the O_2 and pH levels within the retina by O_2, and pH sensitive microelectrodes inserted in the same way as the tungsten electrodes used for recording the nerve impulses from the ganglion cells. The pars plana insertion can be used with an electrode configuration similar to those described above.

Two steps are necessary to make spatiometric recordings of O_2, and pH levels reflect the alterations in oxygen consumption in the retina. The measuring electrode must be placed at the retinal-vitreous interface. The O_2 and the pH level recorded at that location will reflect the O_2 flux across the retina from the various sources.

Secondly, the oxygen level must be correlated with oxygen consumptionby elevating the oxygen levels in the choroid to produce tissues O_2 levels above those in which retinal vessels will release their bound oxygen from hemoglobin. In cats, this is possible by using a breathing mixture with greater than 70% O_2 in it. Alternatively, the retinal circulation can be temporarily interrupted by pressure on the retinal arteries and veins to ensure that all the oxygen measured at the electrode comes from the choroid. Since the oxygen level of the choroid is known, the oxygen level at the retinal vitreous interface allows the computation of the oxygen consumption of the intervening retina and pigment epithelium. The consumption of the inner and outer levels can be separated from each other by placing the electrode in the retina, and also, by changing the retinal illumination from bright to to dark, which changes the O_2 consumption of the receptors as explained below.

The oxygen consumption of the retina changes markedly with the level of illumination (Stefansson et al, 1981 b). This may be due to the receptor metabolism being much higher in the dark than in the light. The changes in the oxygen level following laser photocoagulation curve can indicate:

a) the amount of receptor damage,

b) other changes that may have taken place in the metabolism in the inner layers of the retina, such as in the inner

plexiform layer as may follow exposure to speckle pattern (Zwick and Beatrice, 1978).

5. Animal Procedures and Surgery

Ether was used to induce and maintain anesthesia during stabilization of the eye on a ring sutured to the sclera between the extraocular muscles. In addition, a subcutaneous injection of a local anesthetic was used on all incision sites before surgery. During all experiments, a general inhalation anesthesia, 70% nitrous oxide/30% oxygen mixture, was used. The inspired PCO_2 was monitored continuously and kept at approximately 4.7%. A gallamine triethiodide, dextrose, and saline infusion was continued i.v. during the recording session to aid in stabilizing and fixing the eye.

Although nitrous oxide, even at high partial pressures, did not produce complete surgical anesthesia (Brown et al, 1927; Venes et al, 1971), it has been shown that 70% nitrous oxide with 30% oxygen produces a high degree of sedation and analgesia in both cats and monkeys. It is an adequate anesthetic where only mildly noxious stimulants are present; for example, the direct electrical stimulation of peripheral nerves at frequencies of up to 3 Hz, or foot pad shocks (Venes et al, 1971). In the present experiments, during all surgical procedures, the animals were under deep ether anesthesia at levels sufficient to terminate spontaneous respiration and require artificial ventilation. All incisions were infiltrated with local anesthetic.

When the surgery was ended, the ether was discontinued, and the 70% nitrous oxide, 30% oxygen was used.

The insertion of the recording microelectrodes through the pars plana involved no pain. It is similar to operations often carried on in humans with only local anesthetic. We monitored the heart rate continuously and increased the level of anesthetic when changes in heart rate were detected that could be associated with pain perception. Nitrous oxide was used, because it has been shown to have only slight effects on evoked CNS responses, as compared to the strong depression induced by other volatile anesthetics and barbiturates (Van Norren and Padmos, 1977). A depressant action in the retina has been seen with some of the other anesthetics as well. Obviously, it is important to minimize drug effects on the CNS when studying the activity of the visual system.

The gallamine triethodide was not required to relax the animal, but it did assist in establishing the high degree of eye immobility required for single cell retinal recordings (Enroth-Cugell and Robson, 1966). It has also been established that gallamine triethodide has no effect on retinal ganglion cell responses in cats and monkeys (Enroth-Cugell and Pinto, 1970). We used a nitrous oxide and gallamine triethodide combination because of these considerations.

The normal body temperature was maintained at all times with a heating pad. Cats were maintained in satisfactory physical condition

this way for 24-48 hours. The iris was dilated and accommodation relaxed with several doses of Duke mix (10% phenyephrine, 0.5% mydriacyl, 1:1) supplied approximately every hour.

All animals involved in this study have been maintained and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" (PHS Publication #80-23, 1980).

D. RESULTS AND DISCUSSION

1. Categories of Retinal Ganglion Cell Responses

Retinal ganglion cells have three major cone inputs, one with a 500 nm peak sensitivity, another at 555 nm, and a third at 450 nm. The most common is at 555 nm which is frequently coupled with the 500 nm peak input. The 450 nm peak cone input is found frequently but does not have the same receptive field structure, as there is never a center periphery antagonism between this category of cone inputs. Receptive fields of retinal ganglion cells are symmetrically located about the ganglion cell body, usually at the site of the recording electrode when the electrode was not at the optic disc.

The most general type of receptive field makeup consists of either an excititory or inhibitory response from a restricted group of cones in the center of the receptive field. In antagonism to this central response is a more diffused input, which is strongest in the center and

falls off gradually over a very large area of the retina. Thus, restricted group excititory (often termed ON-center) response would be contrasted with an inhibitory (OFF-periphery) response. These may have the same or different cone sensitivities. The combinations possible are many, since two or even all three cone types may be represented in the center and both the 555 and the 500 nm peak in the periphery. The most common type of ganglion cell found has the 555 cone both in the center and the periphery. The next most common type has both the 555 and 500 nm cone inputs for both the center and periphery. However, many other combinations are also found (Crocker et al, 1980; Wolbarsht et al, 1985).

A detailed discussion of the possible information content of the many types of ganglion cells in relation to visual perception is not appropriate at this time. However, the common types seem to be the ones modulating both visual acuity and color vision, and thus, are the most important ones in the perception of the visual message. Other types seem chiefly concerned with color contrast information, setting absolute levels of light, color vision, and possibly also with border contrast or small motion detection (Wolbarsht et al, 1985).

Although most of the ganglion cell types have been found and stimulated with the Nd laser at threshold energy levels, the majority have not been held long enough to allow the full experimental protocol, including both sub-threshold and super-threshold level laser exposures.

A large portion of the initial period was used to setup and calibrate the equipment. Subsequent reporting periods yielded the successful recordings. Most of the cells found were in the two common groups with input from the 555 and 500 nm cones only. All the experiments of laser exposures were carried out under light adapted conditions, and thus, no effects of the laser exposure on responses from the rod input during scotopic vision could be tested.

In many of the well characterized cell recordings, the effect of laser radiation at levels sufficient to cause threshold type lesions was to dislodge the electrode from its recording site. However, in the majority of the cases recording continued after the threshold type lesion, either because the electrode was located away from the center of the lesion, i.e., at the optic nerve, or the tissue was sufficiently mechanically supported by the electrode to remain in place. This has limited the number of multiple threshold exposure that can be given in the peripheral portions of a ganglion cell or receptive field except when the recording electrode was at the optic disc.

2. Effects of Laser Stimulation on Retinal Ganglion Cell Responses

The results of a typical recording are shown in figure 1. These data are from a center OFF peripheral ON type cell. Both 500 nm and 550 nm cones furnished the central input, while the peripheral had input from 500 nm cones only, as shown in the upper plot in figure 1. The spatial extent of the receptive field is shown in the lower plot in

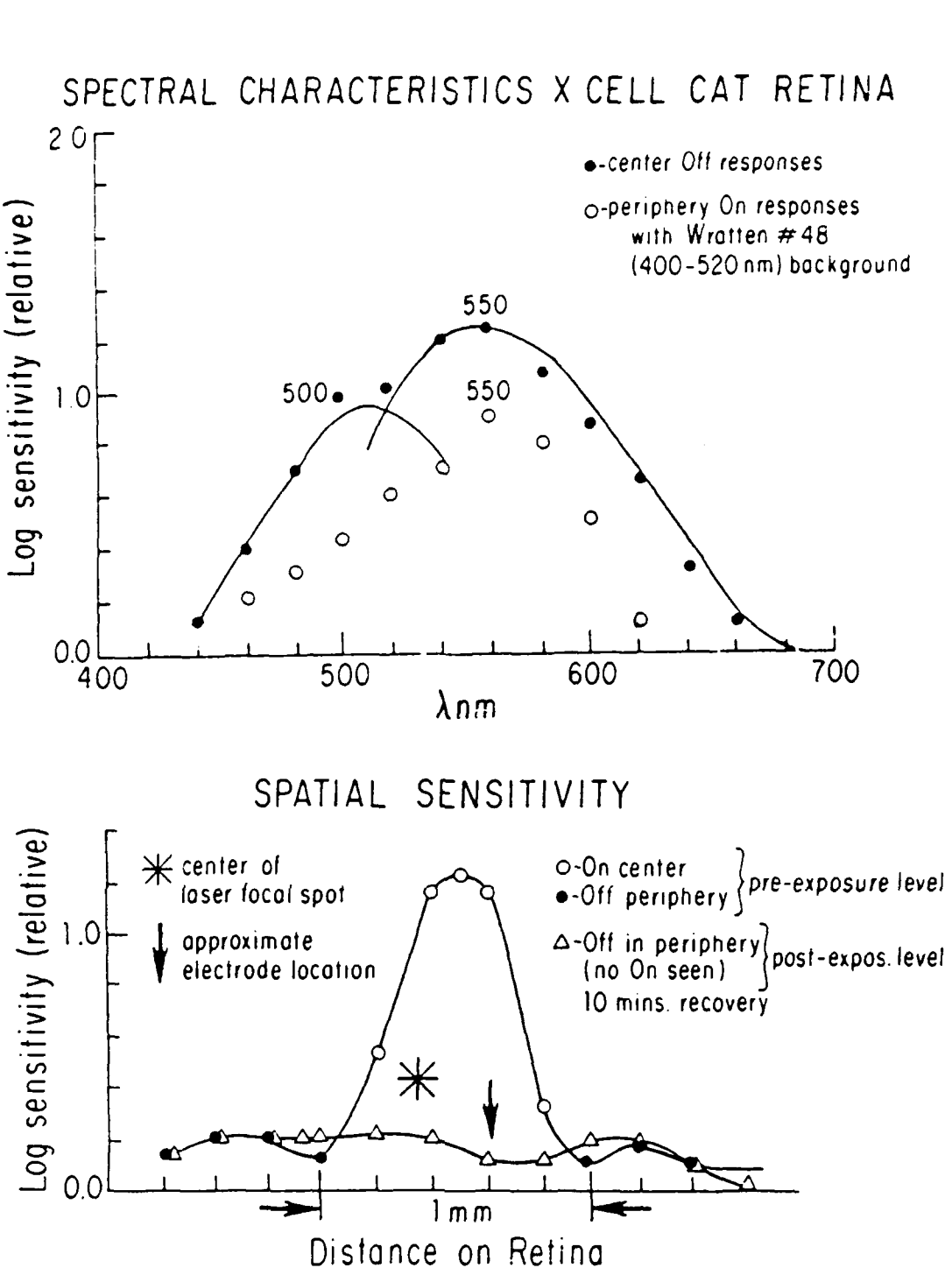

Figure 1. Effect of Nd laser exposure on retinal ganglion cell receptive field sensitivity. The laser exposure was 1,060 nm, 25 ns duration, and about 50 uJ into the eye, with a minimum image size. See text for additional details and interpretation.

figure 1. The central OFF sensitivity covers an area slightly less than 1 mm in diameter, while the peripheral sensitivity (which also extends through the center) has a very large area, perhaps as large as the rest of the retina. Following a threshold type lesion within the receptive field (near the center), there remains only a flat sensitivity to both ON and OFF in the central portion of the field. The electrode in this case was at the center of the receptive field, but recordings continued undiminished during and after the laser exposure, although this type of recording was often ended by electrode movement during laser exposure.

The neural responses to the laser exposure were similar to those of a stimulus at 550 nm which is approximately 50 to 100 times threshold. The latency was short, 3-5 ms, and the bunching of the nerve impulses were similar to that for a 3 ms pulse at 550 nm at 50 times threshold. This large response in this case was followed by a 3 minute (192 sec) period without any responses. Most of this could have been due to light adaptation, as the test stimulus strength was increased only by a factor of 100 (2 log units).

Sub-threshold type exposures of the retina to the focal Nd-glass laser energy (Q-switched with a 20 to 30 ns duration) do not produce long term suppression of function in peripheral zones of the receptive field, other than that due to a decrease in sensitivity which we associate with light adaptation. In all cases, each laser exposure was followed by a massive response by the retinal ganglion cell when the receptive field center was not coincident with the center of the

lesion. Depending upon the type of ganglion cell field makeup, this response was either an excitation (ON response) with a latency of perhaps 5 to 10 ms, or as a rebound from inhibition (OFF response) within perhaps 50 ms. Neither latency changed markedly as the threshold value for injury was approached. The sub-threshold types of exposures caused nearly the same type of response as in ganglion cells eccentric to the site of the lesion. The changes in response sensitivity as a function of position within the field are from a typical cell as shown in figure 1, both before and after laser exposure.

In the cell shown in figure 1, the receptive field center was in the zone where the laser was imaged, and neural function was interrupted to some extent. However, in most, if not all cells, the receptive fields including the peripheral portions are always much larger than the area of the destroyed retina. In the few cases that been studied this way with central lesions, the cells continued to operate, although with a marked reduction of sensitivity in the central portion of the receptive field. That is, in the region of the lesion there was a marked reduction in the amplitude (number of nerve impulses) of the response. Possibly in these cells, as well as the ones illustrated in figure 1, the responses did not go to zero because of scattered light to peripheral portions of the receptive field. This contribution from scattered light may have even increased after the lesion because of local edema and whitening of the retina. However, no

markedly super-threshold (large area) lesions were followed by successful recordings, especially when there was hemorrhage.

The thirty-two cells completely characterized and tested in this fashion had the following distribution:

Two were X type cells with 556 nm cone input to both the center and surround. Six of the X cells also had 500 nm input in the periphery. Five X cells had 450 nm inputs which included two that also had 500 nm input.

There were nine Y type cells, all with 556 nm center and surrounds. Six also had 500 nm in the surround and three had 500 nm cone input in both the center and surround. One also had 450 nm cone input in the center.

Two W type cells were exposed. Both had center surround organization with the 556 nm center in opposition to a 500 nm cone input.

Twenty-one of the cells (15 X, 4 Y, and 2 W types) had exposures below the lesion threshold and showed only light adaptation. A vigorous response was found to the laser exposure in each case, although those cells were all lost when the laser exposure were increased to lesion threshold values. The remainder of the cells

showed losses similar to Figure 1 with central suppression or loss of input. Thus, only the peripheral type of response was seen.

As explained in the methods section, no test animals were produced by exposure to coherent light spectral patterns from an argon laser (488-514 nm) that met the criteria established by Zwick et al (1980) with retinal changes sufficient to cause absence of certain components of the electroretinogram. Also, the cat retina may not have the same sensitivity as the rhesus monkey or the turtle retinas used by Zwick et al (1980). Electroretinographic tests of the exposed cats do not reveal any difference between the exposed animals and unexposed ones. Ganglion cell recordings from the animals exposed to lesser amounts of radiation, as explained in the methods section (approximately 1/5th of the amount required by Zwick et al , 1980), showed no changes in retinal organization as evidenced by the seven ganglion cell responses in the two animals. The center surround organization was normal with approximately the same distribution of cells divided between X and Y types (5 and 2 respectively). If differences were caused in type W cells, this could not be documented, as none of these cells were found in the two animals tested in this fashion.

The acute coherent light speckle pattern exposure from the HeNe laser (694.3 nm) also showed no special effects different from control exposures to red light of the same wavelength. Both types of exposure produced a profound light adaptation, but there were no changes seen in

the local ERG and no effects in the neural responses other than those expected from this amount of light adaptation.

3. Recovery Phenomena of Retinal Ganglion Cells

No retinal ganglion cells were held for more than one hour after laser exposure, and thus, long term follow-up was not possible. In previous similar types of experiments, retinal ganglion cells have been held for as long as twelve hours. Nevertheless, only short term recovery has been observed during this series of tests. However, the last series of experiments seemed to give progressively longer periods of time to accumualte data from each of the cells. This leads us to expect any future experimental programs will have longer recording times.

The short-term recovery seen is, as described above, characteristic of light adaptation. However, where sensitivity has been markedly depressed, as in ganglion cells which have a receptive field coaxial with the site of the laser lesion, it would be helpful to have longer term recovery data to show both the development of injury processes, i.e., edema and repair processes. Also, longer term visual system recovery processes due to neural adaptation and regeneration of photopigment after bleaching could be observed in detail with longer recovery times.

4. Measurement of the O_2 and pH Levels in the Retina

Attempts to measure the retinal O_2 and pH level were unsuccessful in the present series of experiments. Previous experiments had shown that large suprathreshold retinal injuries in which large numbers of the photoreceptors are destroyed produce easily detected changes in available O_2 level at retinal-vitreous junction when the animals are breathing 100% O_2 as opposed to 20% O_2. In the present series of experiments, however, the anesthetic prevented this type of experiment from being performed. The 70% nitrous oxide and the 30% O_2 mixture used was seemingly equivalent to the 100% O_2 in the control experiments as far as the retina was concerned, and no changes were seen when shifting from nitrous oxide/O_2 mixture to the 100% O_2 for short periods of time such as were seen in previous experiments with room air versus 100% O_2 (Stefansson et al, 1981a). This was an unexpected result, and it took several experiments before the causes of this effect could be identified. By the time it was clear what was blocking the unexpected change of O_2 potential even over well fairly visible large lesions or acumulations of small lesions, the experimental program had been terminated.

It should be understood tnat under normal choroidal O_2 levels, the retinal O_2 is maintained at approximately the same level over a laser lesion as in the normal retina. However, where the oxygen level in the choroidal system is increased, then in areas where laser damage is present, the available O_2 will increase due to lower or consumption of this portion of the retina. It was not, however, possible to measure

this change in the present series of experiments due to the type of anesthesia used, so the combined electrophysiological and O_2 level experiments were not possible. In the present series of experiments, O_2 measurements were made only on the animals in a protocol which required a 70% nitrous oxide and 30% O_2 breathing mixture.

pH measurements were attempted at the same time as the O_2 measurements. The resting pH of the surface of the retina was approximately 7.1 to 7.2. No changes were seen at the surface of the vitreous irregardless of the amount of retinal damage. It is possible that the nitrous oxide interfered with the pH electrode, although tests in vitro did not seem to indicate any change in electrode sensitivity in the presence of a nitrous oxide bubbling through arranged test solutions of different pHs from 6.5 to 7.4. Attempts to measure inter-retinal pH potential with the available pH electrodes were unsuccessful. The pH electrode apparently was too blunt to successfully penetrate the internal membrane without grossly damaging the retina. Attempts to insert these electrodes in places where successful penetrations of the internal limiting membrane had been accomplished by sharpened tungsten electrodes were likewise unsuccessful, possibly due to a lack of precision in positioning the pH electrode exactly at the same place where the penetration with the tungsten electrode had been made. The penetrations were attempted under microscopic observation, and the surface of the retina was seen to be grossly depressed. In general, the penetration attempts were halted before severe damage had been done to the retina because of the possibility of displacing the whole eye or causing massive hemorrhage

to terminate the ganglion cell responses recordings in other parts of the retina. A supply of calibrated pH electrodes that had better tip shape and possibly would have been more suitable was only available after the experimental program had been halted. Future experiments may be more successful than the present series in finding any pH changes in the region of laser injury.

E. CONCLUSION AND SUGGESTED FUTURE EXPERIMENTS

The work reported to date has not given as comprehensive testing of the various ganglion cell types, as it represents only the initial portion of the multi-year planned research. As has been described in the various Quarterly Reports (Appendix), the research was somewhat hampered by plans for moving the laboratory and the termination of the program before later phases could take advantage of the large training program that was involved in completing the initial phase of the multi-year proposed protocol. If such a program resumes again, it should be continued on the lines described in the original proposal. Also, attempts should be made in the future to secure more of the nerve fiber recordings at the disc. Although these recording situations are not too hard to achieve, it is difficult to get those fibers connected to a part of the retina that can be reached effectively with the visual stimulator and laser beam. More attention to the anatomy of the eye and the path of the optic nerve fibers in this particular portion of the retina will be helpful in allowing us to get usable optic disc

recordings with greater frequency. It can be concluded, however, that on the basis of the data accumulated to date that a neural message leaves the retina after each sub- or super-threshold laser exposure.

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G. APPENDIX

ARMY CONTRACT # DAMD 17-84-C-4011
Report on Quarterly Period Ending March 1, 1984

The initial part of this period was devoted to equipment set-up and calibration. Primary experiments were run to test the equipment and animal life-support systems. Some minor difficulties were experienced in laser operation and optical system alignment. However, by the end of the period, all the equipment was functioning properly and the first tests with animals showed life-support systems functioning properly. Although several ganglion cells were isolated, none were held long enough to test for operating properties or to expose to laser radiation. The next period will be devoted to increasing the time and stability of the animal operation. No serious difficulties were encountered and progress during this period was approximately that planned during preliminary scheduling. The test neodymium glass Q-switched laser with a 50 ns pulse was measured at approximately 1 joule at 1,064 nm. After frequency doubling to 532 nm, over several milijoules could be achieved with mode selection. This was sufficient for our breakdown, thus sufficient energy was available for all of the planned experiments.

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ARMY CONTRACT #DAMD 17-84-C-4011
Report on Quarterly Period Ending June 1, 1984

During this period, no equipment difficulties were experienced and progress was made approximately along planned lines. Animal support systems continued working without difficulty and we were able to maintain the animals in good physiological condition 48 hours or more while individual cells were kept for 2 hours or more on the average. Characterizations of these isolated cells were improved in order to determine the cell type by linearity and spectral tests in order to judge if the ganglion cell was the type to project the cortex and, thus, be involved in perception in keeping with the overall experimental plan to test perception of a visual stimulus from a laser pulse causing retinal injury..

During this period, the date for the University scheduling of a planned move to new spaces was decided. Construction for the new laboratory spaces was begun. Unfortunately, this has resulted in some diversion of effort from the present planned project, necessitating a new planned contract completion date. At present, the move is scheduled to take place in September. However, the experimental program at the present is still proceeding on a full time basis as far as data collection goes, although analysis of the experimental data has fallen behind.

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DEPARTMENT OF PSYCHOLOGY

Visual System Neural Responses to Laser Exposure from Focal Q-Switched Pulses and Extended Source CW Speckle

Contract DAMD17-84-C-4011

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Interim Report 14 January 1985

Prepared for Department of the Army SGRD-ULZ-RC, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129

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A. BACKGROUND.

Several laser workers have complained that they have received laser exposures resulting in injury although they received little or no visual sensation at the time of the exposure. They complained that they did not realize that they had been exposed at all to a damaging abnormally high level of laser energy and that there was no uncomfortably bright light which made them realize that they had been potentially injured. Documentation of this fact by published reports is difficult, as most such injuries are not given any formal type of publicity due to legal considerations. However, such anecdotal reports that do exist are sufficient to define the problem clearly. Many of the laser injuries in question occur from exposure to energy in the infrared region. Nevertheless, even in this region, the threshold for visual stimulation for laser exposures is still below that for injury. For example, Nd:YAG laser exposures at 1,064 nm give clearly discernible chromatic visual responses at power levels far below those necessary for damage (Sliney et al., 1976). However, some injuries have followed multiple pulse IR exposures in which no single pulse was above the threshold, but as the deleterious effects of multiple pulses in a train are additive, exposure to the entire train results in a suprathreshold level followed by retinal damage. In this case, the last pulses in the train may not have been visible as the injury threshold was reached. This loss of retinal response may have occurred in the damaged area as well as in the surrounding non-damaged area where the receptors are certainly stimulated by subthreshold direct exposure, or scattered light if not directly within an undamaged area.

It is also possible that the peculiar features of the speckle in a sub-injury threshold laser image may produce a chronic inactivation of the retinal response to prevent subsequent appreciation of a supra threshold exposure. Zwick and Beatrice (1978) and Zwick et al. (1980) have indicated that exposure to speckle pattern at levels below those causing thermal damage results in a more or less permanent loss of chromatic sensitivity. This damage seems to be localized in the inner layers of the retina, as the ERG changes which accompany this loss (Zwick et al. 1978, Zwick and Jenkens, 1978) are characteristic of changes in Mueller cell behavior (Karwoski and Proenza, 1977). Zwick and coworkers used laser exposures that included only extremely large retinal areas. Nevertheless, the same type of retinal inactivation may occur on a short term basis in restricted retinal areas. This effect could lower the sensitivity locally enough to prevent appreciation (perception) of many of the final pulses in a laser stimulus train.

A few case reports can be documented in which the retinal injury was not associated with light perception.

Boldrey et al. (1981) reported that an exposure from a Q-switched neodymium YAG at 1.06 um with a 6 ns pulse and 10 pulses per second produced a retinal injury. The patient felt a pop and pain, but did not have any sensation of light. It should be noted that subthreshold neodymium laser pulses at 1,064 nm are reliably seen by almost all observers. For example, Sliney et al. (1968) conducted a series of experiments on the perceived color of this laser exposure. Although many people saw it as green, some saw it as colorless, yet all saw it at approximately the same exposure level.

A retinal injury was caused by exposure to a collimated 585 nm dye cell shifted, frequency doubled Nd-YAG laser with 10 ns pulse repeated at 10 per second. The patient reported that he saw no light flash, but suddenly noticed loss of vision in one eye while the laser was operating (Sliney/NRL-1981).

As mentioned above very few laser accidents are reported in the open literature in detail. Although many semi-public inquiries are made in the course of investigations concerning laser accidents, the possible litigation associated with almost all accidents prevents most cases of laser exposure involving functional loss (i.e. suprathreshold burns) from appearing in the open literature. Most of the reports mention pain following an exposure from a red or infrared laser, and show evidence of a retinal hemorrhage from the description of floaters, etc. (see Boldrey et al., 1981 for review). Although in all cases there is some form of scotoma, this may or may not be associated with a persistent after-image.

The material from clinical laser exposures may be helpful, also. The reports from patients undergoing panretinal photocoagulation are varied. The unsolicited description by the patient is either of pain or no sensation at all other than a flash of light. Pain is felt by relatively few from an argon laser, but much more often from the 670 nm krypton laser treatment. Also, the xenon arc photocoagulator, which has a large retinal image with a large total amount of heat, often has pain reported. However, in the clinical situation, the blink reflex is inhibited and the iris is immobile, eliminating iris spasm, often the cause of the pain following bright light exposure. When the pain from this cause is eliminated, the light may not seem as bright or even may not be objectionable.

The halo surrounding the control portion of a diffraction limited image of a laser contains a considerable portion of the energy of even a threshold exposure. Even if the energy in the first diffraction ring is only 5% of that in the center, this should be sufficient to give a visual stimulus to those receptors which surround the laser damaged group in the center. Since the outer segments of the receptors are not exactly in the focal plane of the pigment epithelium which is the thermal heat source, some out of focus broadening of the damage zone may be expected. Nevertheless, even outside of the approximately 0.02 mm retinal lesion formed by a diffraction limited image laser exposure, there is a large retinal area illuminated by at least 1,000th of the peak intensity in the center. This level is certainly high enough to produce visual perception. As this area would be increased by any scattered light, it seems clear that a sizeable, non-injured group of photoreceptors are stimulated sufficiently to give a visual response during any damage level exposure.

Several factors could prevent the information from leaving the retina along the optic nerve fibers after stimulation of the receptors. For example, in the macular region, if the laser is the proper wavelength to be absorbed by the macular pigment, xanthophl a lesion can be formed in the outer plexiform layer at the same time and at the same power level as a lesion is formed in the photoreceptor outer segments and pigment epithelium. The blue line, 488 nm from an argon laser will cause this kind of double lesion (Wolbarsht and Landers, 1972; Landers et al., 1976, and Boybyes et al., 1973). If such a lesion in the inner retina was to form quickly enough, it would block the flow of information

from receptors immediately underneath. Each ganglion cell is connected to a large number of receptors through a large and complex receptive field. However, both the size and complexity of the receptive fields of most ganglion cells will still allow stimulation of ganglion cells in the damaged areas by peripheral receptors. The diffuse organization of the retina will allow information flow to proceed so that the message from the receptors can reach the optic nerve and be transmitted back to the brain, initiating perception. Nevertheless, it seems that a short laser pulse would act as a stimulus source for all receptors receiving sufficient photons. This kind of a stimulus will activate later neurons because of the large change in stimulus strength, although the stimulus parameters might not be accurately described, especially with respect to color. It seems reasonable on the basis of our present knowledge that the initial stimulus (in addition to some kind of afterimage) would certainly be perceived from a short laser pulse. Possibly a long duration laser pulse could be delivered with a proper time temperature histogram to cause the receptors to die at the same time they were stimulated without, however, exceeding the visual threshold in some (but probably not all) surrounding receptors. This would be a minimal visual stimulus case.

Numerous studies have indicated that there are detectable changes in the retinal anatomy from pulsed laser exposures in the infrared below those giving ophthalmoscopically visible loss of function. The widespread use of many kinds of laser equipment operating in the near infrared in multipulse modes could result in large amounts of subthreshold laser exposures to Army personnel. The work of Adams et al. (1972), has shown that minimal pathological changes in the retina can result from single pulsed laser exposures. Although this type of minimal damage is far below that demonstrated to result in a functional loss, it is possible that the cumulative results of such exposures might result in physiological changes in the eye, ultimately leading to functional loss.

The physiological changes resulting from suprathreshold laser exposures indicate alterations in distribution of oxygen tension within the retina following laser exposure due to changes in metabolism in the various layers of the retina, as for example, in therapeutic laser photocoagulation which results in widespread destruction of the photoreceptor layer allowing choroidal oxygen to diffuse into the inner layers of the retina (Wolbarsht and Landers, 1980). It is possible that even without widespread destruction of the photoreceptor layer, multiple exposures might lower the metabolic activity of the photoreceptor cells, the rods, and cones, and compromise their metabolism, resulting in a lower rate of oxygen consumption. This would allow additional oxygen to diffuse into the inner retinal layers. Autoregulation of tissue oxygen levels would then reduce the retinal circulation flow rate which could result in a buildup of metabolic waste products, CO_2 and lactic acid, and thus, could lower the normal pH, possibly leading to the degeneration of the inner layers of the retina as seen in retinitis pigmentosa (Wolbarsht & Landers, 1980; Stefansson et al., 1981 a). Those are all long term effects and if they occurred at all would not be quick enough to change the visual perception of an isolated focal laser exposure.

The changes in ERG responses mentioned by Zwick and Beatrice (1978), from speckle pattern may have a similar chronic degenerative effect on the inner retina, although in different cell groups as indicated by the ERG changes (Karwoski and Proenza, 1977). This whole effect may be due to increased synaptic activity in the inner plexiform layer due to the speckle pattern. This activity may lead to metabolic changes and, if continued, pathological ones.

B. PRESENT WORK

The site of a laser burn in the pigment epithelium and adjacent photoreceptors and the relative circulatory and metabolic interdependence of this part of the retina from the innerneural layers both suggest that even the destruction of the photoreceptors will not disturb the function of the overlying neural retina. It is also suggested that sufficient diffracted or scattered laser radiation in the portion of the retina surrounding the thermally damaged sufficient scattered light will generate a neural signal following the laser exposure. This information will be passed on through the inner retinal neural layers up into the optic nerve and on to the higher visual centers. Thus, it is almost certain that perception of a laser exposure will take place and that the perception will be of a source larger than a diffraction limited source used to produce the lesion. Nevertheless, this signal may be too weak to be seen in the presence of any significant ambient light. In the present series of experiments measurements of the neural function have been used to test whether in fact the retinal neural layers are stimulated, and to show that a signal about the actual laser exposure reaches the retinal ganglion cells and is passed on to the higher visual centers thus indicating that perception of a laser stimulation has taken place.

Even though the visual sensitivity is low wavelengths of 1,060 or 1,064 nm from the neodymium glass or YAG lasers, nevertheless, the diffraction limited image of this source does produce sufficient energy away from the injury zone of the diffraction image to give significant visual information of the laser source. In the present series of experiments the latency of the ganglion cell response has been measured following the almost certain death of the photoreceptor cells in the central of the lesion.

METHODS

External recordings from retinal ganglion cell bodies and nerve fibers of the optic disc have been used to monitor the exposure senstivity of the retina in the position of the laser image and to document the exposure conditon which caused certain types of response. Intraocular metal electrodes have been used to monitor the nervous activity in the retinal ganglion cells. The structure of the receptive field of the ganglion cells have been mapped using the techniques of Wagner et al. (1960), and Wolbarsht et al. (1985). Responses to test subthreshold laser exposures have been determined. Both subthreshold and markedly superthreshold exposures have been used to determine whether markedly super-threshold exposures inhibit information flow and thus give a poor or absent perception of laser exposure when threshold level laser exposures give an unmistakable signal.

The present series of exposures have only been done with single Q-switched laser pulses. The cumulative effects of multipho pulse have not, as yet, been tested.

1. Laser Exposure and Optical Stimulation.

Optical stimulation has been provided by a Maxwellian view system adapted from a two independent channels system similar to that described by Wagner et al. (1960) modified with interference filters substituted for the monochrometer as subscribed in Crocker et al. (1980). The two channels have been used with

either interference filters or broadband gelatin filters (Wratten Type Eastman Kodak Company, Rochester, New York) to furnish chromatic adaptation as used in identification of the type of the ganglion cells and characterization of the receptive field properties. The beams from the two channels can be combined so that they can pass as a single beam to a flat face corneal contact lens in the center of the fully dilated animal pupil. In this configuration, a focused, demagnified (10 to 1) image of the optical stimulator apperture is formed on the retina. Under these conditions approximately 10^{o} either side of center of the image of the beam can be stimulated on the retina directly. The selected retinal ganglion cells have been identified before and after subthreshold laser exposures to determine how the response has changed following a superthreshold laser exposure. Maxwellian view optics have been used to allow sufficient intensitiy of chromatic narrow-band stimulating light to characterize easily and quickly the performance of selected ganglion cells to a high degree of precision. The field apperture of the optical stimulator can form an image on the retina as small as 0.02 mm. Previous work has indicated that this is a very reliable figure as it has been compared with known size of probes inserted into the eye and placed at the same place as the test pattern on the retina. Both laser exposure and stimulus beams have been approximately normal to the retina eliminating any change in stimulus response from the Stiles-Crawford effect. In order to achieve the Maxwellian view, a flat face contact or (gonial) lens has been used on the cornea to eliminate that refracting surface and to assist in visualizing the posterior pole of the eye for long periods of time. A flat face soft contact lens which is highly oxygen permeable was used. Flowing well oxygenated solutions around the edge of this soft contact lens insured that the cornea remained clear during the course of the experiment.

The laser beams for lesion formation were introduced through a third channel and located and focused with a coaxial, low power (0.5 mW) helium-neon laser indicator beam attenuated in the minimum stimulus. The neodymium-glass laser system used for lesion formation has a 0.5 cm x 7.5 cm neodymium-glass and is Q-switched. It has a primary wavelength of 1,060 nm and can be frequency doubled. The beam is 5 mm in diameter at the cornea, and thus no artificial pupil is needed. In the present series of experiments mode selection has not been used to give the minimum size image, but beam expansion and written with mode selection by apperture position is planned for future experiments.

The calibration has been carried out with a calorimeter and a PIN photodiode to show the shape of the pulse. The absence of troublesome hot spots have been examined by photographing the raw beam.

2. Electrophysiological Techniques

Extracellular recordings will be made from retinal ganglion cells and optic nerve fibers from the intact eyes of adult cats, and cynomologous monkeys. The electrophysiological recording equipment has been described previously in detail (Crocker et al., 1980).

Tungsten wire, electrolytically sharpened electrodes similar to those described by Levick (1972) will be used in conjunction with a FET amplifier input stage. The amplified signal will be put through a delay line filter to allow discrimination of two or more kinds of impulses, so that different ganglion cells can be used simultaneously for testing. However, in most cases, responses from single cells will be isolated. The electrode and carrier will be

inserted into the eye through the region of the pars plana, as in conventional vitrectomy surgery. The electrode will then be placed against the retina under visual observation through the anterior part of the eye. When the electrode is positioned in the selected part of the retina, it will be moved forward until it just penetrates the internal limiting lamina. Some recordings will be made from ganglion cell bodies or at the near by axon. In this type of recording, most of the electrode positions are at or near the center of the receptive field of the ganglion cell. As there are often mechanical motions of the electrode due to laser exposure, recordings will also be made from the ganglion cell axons where they enter the optic disk. This will be done in the same fashion by advancing the electrode to the rim of the disk. Previous experiments show the recording made at the disk are from the same kind of cells as are found by cell body or nearby axon recording. The recordings at the optic disk are stable and seem capable of continuing from the same neuron after laser exposure to a portion of the retina in the receptive field of that ganglion cell.

However, it is more difficult to find cells at the disk and recording times are much shorter, although some recordings can be made for the duration of several hours. The technique to achieve these long lasting recordings from the disk will be optimized in the future as the data for markedly suprathreshold exposures can be more reliably collected from them.

A. Data Acquisition and Analysis.

The data points will be determined from the initial sensitivity of the ganglion and its other parameters by a constant response technique. This is in order to ensure that we can interpret the given response from the ganglion cell to laser exposure. The ON/OFF response characteristics will be documented as well as the chromatic responses of the cell and its makeup in receptive field and its receptive field organization into center and surround. Both rod and cone contributions will be identified in order to determine, if possible, which of the receptors gives rise to the response to the laser exposure.

3. Measurement of the O_2 and pH level in the Eye

The technique for the measurement of the O_2 potentials has been described in detail previously (Stefansson, et al., 1981; Landers, 1978). The O_2 and pH levels within the retina will be measured by O_2 and pH sensitive microelectrodes inserted in the same way as the tungsten electrodes used for recording the nerve impulses from the ganglion cells. The pars plana insertion will be used, and electrode configuration will be similar to those described above.

The two steps are necessary to make spatiometric recordings of oxygen levels and pH levels reflect the alterations in oxygen consumption in the retina. The measuring electrode is placed at the retina vitreous interface. The O_2 and pH level recorded there will reflect the flux across the retina from the various sources. The oxygen level can be correlated with oxygen consumption by averaging the oxygen levels in the choroid to produce oxygen levels in the tissues above those in which retinal vessels will release their bound oxygen from hemoglobin. Alternatively, the retinal circulation can be temporarily interrupted by pressure on the retinal arteries and veins to ensure that all the

oxygen measured at the electrode comes from the choroid. Since the oxygen level of the choroid is known, the oxygen level at the retinal vitreous interface allows the computation of the oxygen consumption of the intervening retina and pigment epithelium. The consumption of the inner and outer levels can be separated from each other by placing the electrode in the retina; also, by changing the stimulating condition from light to dark, which changes the O_2 consumption of the receptors.

The oxygen consumption of the retina changes markedly in light and dark (Stefannson et al., 1981 b). This may be due to the receptor metabolism being much higher in the dark than in the light. The changes in the oxygen level following laser photocoagulation curve can indicate:

1) the amount of receptor damage,
2) other changes that may have taken place in the metabolism in the inner layers of the retina, such as in the inner plexiform layer as may follow exposure to speckle pattern (Zwick and Beatrice, 1978).

4. Animal Procedures and Surgery

Ether was used to induce and maintain anesthesia during stabilization of the eye on a ring sutured to the sclera between the extraocular muscles. In addition, a subcutaneous injection of a local anesthetic was used on all incision sites before surgery. During the experiment, a general inhalation anesthesia, 70% nitrous oxide/30% oxygen mixture, was used. The inspired PCO_2 was monitored continuously and kept at approximately 4.7%. A gallamine triethiodide, dextrose and saline infusion was continued i.v. during the recording session to aid in stabilizing and fixing the eye.

Although nitrous oxide, even at high partial pressures, did not produce complete surgical anesthesia (Brown et al., 1920; Venes et al., 1971), it has been shown that 70% nitrous oxide with 30% oxygen produces a high degree of sedation and analgesia in both cats and monkeys. It is an adequate anesthetic where only mildly noxious stimulants are present; for example, the direct electrical stimulation of peripheral nerves at frequencies of up to 3 Hz or foot pad shocks. (Venes et al., 1971). In the present experiments, the animals were under deep ether anesthesia during all surgical procedures at levels sufficient to terminate spontaneous respiration or require artificial ventilation. All incisions were infiltrated with local anesthetic. When the surgery was ended, the ether was discontinued, and the 70% nitrous oxide, 30% oxygen was used.

The insertion of the recording microelectrodes through the pars plana involved no pain. It is similar to operations often carried on in humans with only local anesthetic. We monitored the heart rate continuously and increased the level of anesthetic when changes in heart rate were detected that could be associated with pain perception. Nitrous oxide was used because it has been shown to have only slight effects on evoked CNS responses, as compared to the strong depression induced by other volatile anesthetics and barbiturates (Van Norren and Padmos, 1977). A depressant action in the retina has been seen with some of the other anesthetics as well. Obviously, it is important to minimize drug effects on the CNS when studying the activity of the visual system.

The gallamine triethodide was not required to relax the animal, but it did assist in establishing the high degree of eye immobility required for single cell retinal recordings (Enroth-Cugell and Robson, 1966). It has also been established that gallamine triethodide has no effect on retinal ganglion cell responses in cats and monkeys (Enroth-Cugell and Pinto, 1970). We used a nitrous oxide and gallamine triethodide combination because of these considerations.

The normal body temperature has been maintained at all times with a heating pad. Cats were maintained in satisfactory physical condition this way for 24-48 hours. The iris was dilated and accommodation relaxed with several doses of Duke mix (10% phenyephrine 0.5% mydriacyl, 1:1) supplied approximately every hour.

All animals involved in this study have been maintained and used in accordance with the Animal Welfare Act of 1970, and the "Guide for the Care and Use of Laboratory Animals" (PHS Publication #80-23, 1980).

RESULTS

1. Categories of Retinal Ganglion Cell Responses.

Retinal ganglion cells have three major cone inputs, one with a 500 nm peak sensitivity, another at 555 nm, and a third at 450 nm. The most common is at 555 nm which is frequently coupled with the 500 nm peak input. The 450 nm peak cone input is found rarely and does not have the same receptive field structure. Receptive fields of retinal ganglion cells are symmetrically located about the ganglion cell body, usually at the site of the recording electrode.

The most general type of receptive field makeup consists of either an excititory or inhibitory response from a restricted group of cones in the center of the receptive field. In antagonism to this central response is a more diffused input, although strongest in the center, which falls off gradually over a very large area of the retina. Thus, an excititory (often termed ON-center is contrasted with an inhibitory or OFF periphery response. These may have the same or different cone sensitivity. The combinations possible are many since two or all three cone types may be represented in the center, and both the 555 and the 500 nm peak in the periphery. The most common type of ganglion cell found has the 555 cone both in the center and the periphery. The next most common type has both the 555 and 500 nm cone inputs for both the center and periphery. However, many other combinations are also found (Crocker et al., 1980; Wolbarsht et al., 1985).

A detailed discussion of the possible information content of the many types of ganglion cells in relation to visual perception is not appropriate at this time. However, the common types seem to be the ones modulating visual acuity, and thus are the most important ones in the perception of the visual message. Other types seem chiefly concerned with color contrast information, with color vision, and possibly also with border contrast (Wolbarsht et al., 1985).

To date in the present program only a few of these types have been found and stimulated with the Nd laser at threshold energy levels. The majority of the types have only been found and characterized sufficiently well to be identified, but have not been held long enough to allow the full experimental protocol, including both sub-threshold and supra-threshold level laser exposures. The majority of the initial period has been devoted to setup and calibration of the equipment. Subsequent reporting periods have produced more successful recordings. Most of the cells found, however, were in the two common groups with input from the 555 and 500 nm cones only. All the experiments are carried out under light adapted conditions and no effect of the laser exposure on responses from the rod input and scotopic vision were tested.

2. Effects of Laser Stimulation on Retinal Ganglion Cell Responses.

In many of the well characterized cell recordings the effect of laser radiation at levels sufficient to cause threshold type lesions is to dislodge the electrode from its recording site. However, in the majority of the cases recording continues after the threshold type lesion, either because the electrode is located away from the center of the lesion, i.e., at the optic nerve or the tissue is sufficiently mechanically supported by the electrode not to be dislodged. Under these conditions the results based on a preliminary analysis of the data in summary is as follows:

Subthreshold type exposures of the retina to the focal Nd-glass laser energy (Q-switched with a 20 to 30 ns duration) does not produce long term suppression of function in peripheral zones of the receptive field, other than that due to a decrease in sensitivity which we associate with light adaptation. In all cases each laser exposure is followed by a massive response by the retinal ganglion cell when the receptive field center was not coincident with the center of the lesion. Depending upon the type of ganglion cell field makeup, this response was either an excitation (ON response) with a latency of perhaps 5 to 10 ms, or as a rebound from inhibition (OFF response) within perhaps 50 ms. Neither latency changed markedly as the threshold value for injury was approached. The subthreshold types of exposures caused nearly the same type of response as in ganglion cells eccentric to the site of the lesion. The changes in responses from a typical cell are shown in Figure 1 both before and after laser exposure.

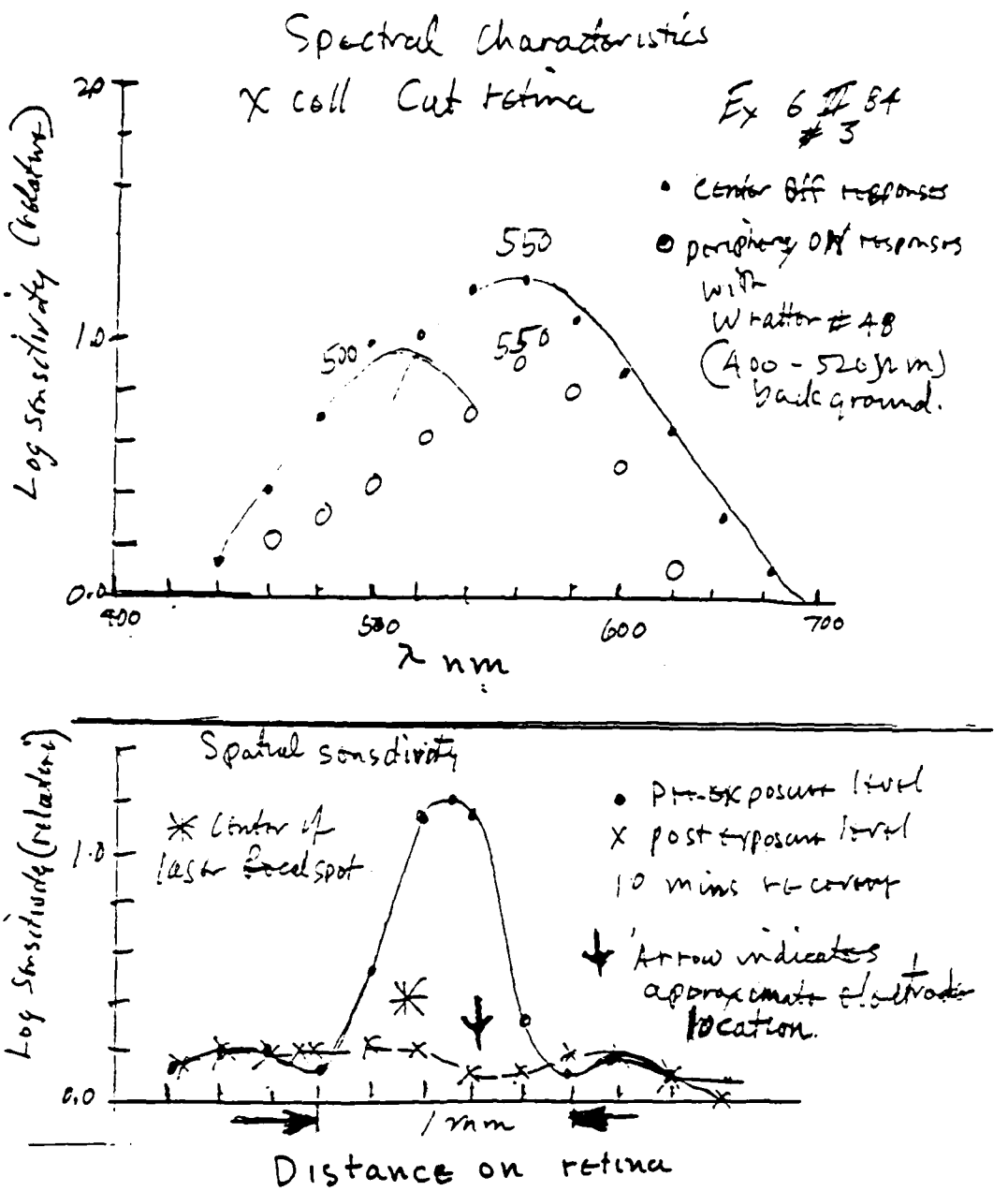

Figure 1. Effect of Nd laser exposure on retinal ganglion cell receptive field sensitivity. The laser exposure was 1,060 nm, ~25 ns duration, and about 50 μJ into the eye.

In the cell shown in Figure 1, the receptive field center was in the zone where the laser was imaged, and neural function was interrupted to some extent. However, in most, if not all cells, the receptive fields including the peripheral portions are always much larger than the area of the destroyed retina. In the few cases that have been studied this way with central lesions, the cells continued to operate although with a marked reduction of sensitivity in the central portion of the receptive field. That is, in the region of the lesion there was a great dimunition of response. Possibly in these cells as well as the ones illustrated in Figure 1 the responses did not go to zero because of scattered light to peripheral portions of the receptive field. This may have increased after the lesion because of local edema and whitening of the retina. However, as yet, no markedly superthreshold lesions have been been followed by successful recordings.

3. Recovery Phenomena of Retinal Ganglion Cells.

To date, no retinal ganglion cells have been held for more than one hour to allow long term followup after laser exposure. In previous use of the equipment in similar types of recording setups, retinal ganglion cells have been held for as long as twelve hours. Nevertheless, only short term recovery has been observed up to the present time. However, in the last series of experiments each recording session seemed to have better success with progressively longer periods of time to accumulate data from each of the cells. This leads us to expect that future experiments will have much longer recording times.

The short term recovery seen is, as described above, characteristic of light adaptation. However, where sensitivity has been markedly depressed, as in ganglion cells which have a receptive field coaxial with the site of the laser lesion, it would be helpful to have longer term recovery data to show both the development of injury processes, i.e., edema, and repair processes. Also, longer term visual system recovery processes due to neural adaptation and regeneration of photopigment after bleaching could be observed in detail.

E. CONCLUSION

The work reported to date has not ~~been given~~ included comprehensive testing of the various ganglion cell types, and represents only the initial portion of the planned research. As has been described in the various Quarterly Reports (attached), the research has been hampered by plans for moving the laboratory. This move is in progress at the present time. However, the new laboratory spaces are equivalent in every way to the old ones and will certainly allow the remainder of the experiments to go forth as planned. When the program resumes again, it will continued on the lines described in the original proposal. Also, attempts will be made in the future to secure more of the nerve fiber recording at the disc. Although these recordings are not too hard to find, it is difficult to get them in a part of the retina that can be reached effectively with the visual stimulator and laser beam. More attention to the anatomy of the eye and the path of the optic nerve fibers in this particular portion of the retina will be helpful in allowing us to get usable optic disc recordings with greater frequency.

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